matter has been added. Applicant respectfully requests reconsideration and allowance of the present application.

I. SEQUENCE LISTING:

Applicant provides herewith a paper copy and computer readable copy of the sequence listing for the present application in accordance with 37 C.F.R. 1.821 et seq.

II. DRAWINGS:

Applicant respectfully requests deferment of the filing of formal drawings until, and at such time, as Applicant receives a Notice of Allowance.

Applicant submits that the drawings comply with 37 C.F.R. §1.83(a).

III. SPECIFICATION:

The Office Action objects to the disclosure because the "Brief Description of the Drawings" allegedly does not reflect the claim language. Applicant respectfully submits that the description of the drawings reflects the pending claim language.

The specification (including the Examples) has been amended throughout to correct typographical mistakes and to clarify abbreviations based upon their first use in the description.

The Office Action alleges that the specification fails to provide proper antecedent basis for the claimed subject matter. In particular, the Office Action alleges that the specification fails to describe or mention "extremophiles", "thermophiles, hyperthermophiles, psychrophiles, and psychrotrophs". Applicant respectfully directs the Examiner to page 16, lines 9-11 which recite these terms.

IV. REJECTION UNDER 35 U.S.C. §112, FIRST PARAGRAPH

with gene expression of a reporter gene as the basis of the detectable signal, the specification

allegedly does not reasonably provide enablement for performing the method in a cell with a non-gene expression based detectable signal or for performing the method *in vitro* with either type of detectable signal. Applicant respectfully traverses this rejection.

The Office Action alleges that the present specification does not enable a non-gene based detectable signal. Applicant respectfully reminds the Examiner that a patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 3 USPQ2d 1737 (Fed. Cir. 1987); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984). All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art.

In vitro gene expression systems or "cell-free" systems have been known for over a decade. Accordingly, one skilled in that art would be able to perform the methods of the present invention in a cell-free system based upon teaching known to those of skill in the art at the time the present application was filed and based upon the teachings provided in the present specification.

In addition, non-gene based detectable signals were known in the art at the time of filing the present application. The specification teaches the quenching effect and fluorescence effect of GFP molecules in close proximity (pages 34-35). One of skill in the art would recognize from the present teaching that rather than utilizing a gene based reporter system, the association (or lack thereof) of two hybrid proteins (*i.e.*, a first protein with a first fluorescent molecule, and a second protein with a second fluorescent molecule) can be monitored due to fluorescent

are associated, there is a quenching of one fluorescent wavelength and an increase in another

fluorescent wavelength. The changes in wavelengths can be monitored by standard optical methods. Accordingly, Applicant respectfully requests withdrawal of the §112, first paragraph, rejection.

Claims 45-47 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention without undue experimentation. The Office Action alleges that claims 45-47 have additional method steps which have no clear connection to the steps preceding A-F and succeeding steps (i)-(ii). While Applicant respectfully traverses this rejection, claims 45-47 have been amended to clarify the relationship of the method based on the sub-numbering. Accordingly, Applicant respectfully requests withdrawal of the rejection.

V. REJECTION UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 16-47 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicant respectfully traverses this rejection.

The Office Action alleges that claim 16 is indefinite because the terms "interaction of a first hybrid protein and second hybrid protein" and "wherein interaction of the first protein and second protein" appear to be different interactions that need to be clarified. Applicant has amended claim 16 to claim the invention with better particularity and therefore respectfully requests withdrawal of the §112, second paragraph, rejection.

The Office Action alleges that claim 17 is indefinite because of the phrase, "the

rejection of claim 17 under 35 U.S.C. §112, second paragraph, is moot.

Claim 36 is allegedly indefinite for recitation of "in the absence . . . of a detectable response". As the Examiner correctly stated, the phrase is meant to refer to the "off" or "negative" state of a binary signal response (*e.g.*, repression of gene expression). However, in contrast to the Examiner's statement that claim 36 is "essentially a duplicate of claim 16", Applicant respectfully submits that claims 16 and 36 are not duplicative but claim the subject matter of the present invention in patentably different ways (e.g. claim 36 includes a contacting element not recited in claim 16). Accordingly, Applicant respectfully requests withdrawal of the §112, second paragraph, rejection.

Claims 22-25 and 38-40 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly incomplete for omitting essential steps. The Office Action alleges that the omitted steps are "expression of the first, second and third recombinant genes in the host cell which is requisite for the method to succeed." (Office Action at page 8, paragraph 16.) Applicant respectfully traverses this rejection.

Applicant respectfully submits that the rejected claims do not omit an essential step. For example, claims 38-40 recite providing a first, second or third gene expressed in the host cell. Accordingly, Applicant respectfully requests withdrawal of the §112, second paragraph, rejection.

Claims 45-47 stand rejected as allegedly indefinite for recitation of additional method steps which are not explicitly related to the method steps cited in claims on which they are dependent. Applicant respectfully traverses this rejection.

Claim 45-47 have been amended to clarify the relationship of the sub-numbering recited in the claims. Accordingly, Applicant respectfully requests withdrawal of the §112,

VI. REJECTION UNDER 35 U.S.C. §102

Claims 16-26 and 36-42 stand rejected under 35 U.S.C. §102 as allegedly anticipated by U.S. Patent No. 5,525,490 to Erickson *et al.* Applicant respectfully traverses this rejection.

Erickson *et al.* allegedly discloses a method for identifying a molecule which modulates the interaction between at least a first and second protein. Erickson *et al.*, does not teach or suggest a molecule from a library generated from a mixed population of organisms as recited in Applicant's claims 16 and 36, upon which the remaining claims depend. Erickson *et al.* fails to teach or suggest each and every element of Applicant's invention. Accordingly, Applicant respectfully requests withdrawal of the §102 rejection.

Claims 16-17, 20, 22, 23, 24, and 36-39 stand rejected under 35 U.S.C. §102 as allegedly anticipated by U.S. Patent No. 5,322,801 to Kingston *et al.* Applicant respectfully traverses this rejection.

Kingston *et al.* allegedly discloses a method for identifying a molecule which modulates the interaction between at least a first and second protein. Kingston *et al.*, does not teach or suggest a molecule from a library generated from a mixed population of organisms as recited in Applicant's claims 16 and 36, upon which the remaining claims depend. Kingston *et al.* cannot anticipate Applicant's claimed invention because Kingston *et al.* fails to teach or suggest each and every element of Applicant's invention. Accordingly, Applicant respectfully requests withdrawal of the §102 rejection.

VII. REJECTION UNDER 35 U.S.C. §103

Claims 16-26 and 36-42 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over either Erickson *et al.* or Kingston *et al.* in view of Mendelsohn *et al.* (Curr. Op. in Biotech. 1994 5:482-486). Applicant respectfully traverses this rejection.

Neither Erickson *et al.* nor Kingston *et al.* teach or suggest the claimed invention, as discussed above. Mendelsohn *et al.* also does not teach a molecule from a library made from a mixed population of organisms as recited in Applicant's claims 16 and 36. Thus, even if there were some suggestion to combine Mendelsohn *et al.* with Erickson *et al.* and/or Kingston *et al.*, which there is not, the combination of references does not teach or suggest the use of such a library as a source of DNA in the method of the claimed invention. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

Claims 16-45 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view Stein *et al.* (1996 J. Bact. 178:591-599) and Horikoshi (1995 Curr. Op. in Biotech. 6:292-297). Applicant respectfully traverses this rejection.

Applicant respectfully submits that the Office Action has failed to set forth a *prima facie* case of obviousness. In the absence of Applicant's disclosure, there must be found, at the time of filing, motivation or teaching to combine the cited references. In this case, there is no such motivation outside the disclosure of Applicant's invention. The alleged teaching is found, not in the references, but in the claims being rejected. It is error to reconstruct the claimed invention from the prior art by using the rejected claim as a "blueprint." *Interconnect Planning Corp. v. Feil*, 227 USPQ 543, 548 (Fed. Cir. 1985).

[illegible]

population of organisms, wherein the molecule either directly or indirectly [illegible]

protein interactions, for example. Stein *et al.* allegedly teaches creating libraries from uncultivated marine microorganisms. Stein *et al.* does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Horikoshi allegedly teaches enriching populations of extremophiles for prokaryotic organisms. Horikoshi does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. The combination of the foregoing references fails to provide any motivation or reason, and thus provide the claimed method, to mixed population libraries for molecules that can modulate protein-protein interactions. Applicant respectfully submits that the present rejection is based upon hindsight reconstruction of Applicant's invention based upon a number of references that do not teach or suggest the combination. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

Claims 16-33 and 36-45 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view of Short *et al.* (WO 97/04077) and Horikoshi. Applicant respectfully traverses this rejection.

Erickson *et al.* has been discussed above. Short *et al.* is cumulative to Stein *et al.* above, and allegedly teach creating libraries from uncultivated microorganisms. These references do not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Horikoshi allegedly teaches enriching populations of extremophiles for prokaryotic organisms. Horikoshi does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. The combination of the foregoing references fails to provide any motivation or reason, and thus provide the claimed method, for searching or examining mixed population libraries for molecules that can modulate protein-protein interactions. Applicant respectfully submits that the present rejection is based upon hindsight reconstruction of Applicant's invention

Claims 16-47 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Erickson *et al.* in view of Stein *et al.* and Horikoshi, as applied to claims 16-45, and further in view of Patanjali *et al.* Applicant respectfully traverses this rejection.

Applicant respectfully submits that the Office Action has failed to set forth a *prima facie* case of obviousness. There is no suggestion, teaching, or motivation to arrive at Applicant's invention of identifying molecules in a library made from a mixed population of organisms that modulate interacting molecules. As discussed above, Erickson *et al.* fails to teach or suggest the claimed invention. Stein *et al.* allegedly teaches creating libraries from uncultivated marine microorganisms. Stein *et al.* does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Horikoshi allegedly teaches enriching populations of extremophiles for prokaryotic organisms but does not teach or suggest identifying proteins or molecules that modulate protein-protein interactions. Patanjuli *et al.* is combined with the foregoing references to allegedly teach normalization of cDNA. The addition of Patanjuli *et al.* does not remedy the deficiencies of the prior references and thus does not provide a *prima facie* case of obviousness. The combination of the foregoing references fails to teach the claimed invention. Applicant respectfully submits that the present rejection is based upon hindsight reconstruction of Applicant's invention based upon a number of references that do not teach or suggest the combination. Accordingly, Applicant respectfully requests withdrawal of the §103(a) rejection.

In view of the above remarks, reconsideration and favorable action on all claims is respectfully requested.

Respectfully submitted,

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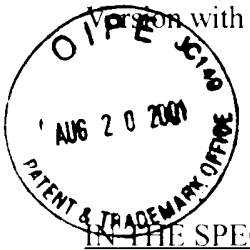

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

The paragraph at page 1, lines 16-27 was changed as follows:

Within the last decade there has been a dramatic increase in the need for bioactive compounds with novel activities. This demand has arisen largely from changes in worldwide demographics coupled with the clear and increasing trend in the number of pathogenic organisms that are resistant to currently available antibiotics. For example, while there has been a surge in demand for antibacterial drugs in emerging nations with young populations, countries with aging populations, such as the US, require a growing repertoire of drugs against cancer, diabetes, arthritis and other debilitating conditions. The death rate from infectious diseases has increased 58% between 1980 and 1992 [(1)] and it has been estimated that the emergence of antibiotic resistant microbes has added in excess of $30 billion annually to the cost of health care in the US alone [(2)]. As a response to this trend, pharmaceutical companies have significantly increased their screening of microbial diversity for compounds with unique activities or specificities.

The paragraph at page 2, lines 12-27 was changed as follows:

The majority of bioactive compounds currently in use are derived from soil microorganisms. Many microbes inhabiting soils and other complex ecological communities produce a variety of compounds that increase their ability to survive and proliferate. These compounds are generally thought to be nonessential for growth of the organism and are synthesized with the aid of genes involved in intermediary metabolism hence their name - secondary metabolites. Secondary metabolites that influence the growth or survival of other organisms are known as bioactive compounds and serve as key components of the chemical defense arsenal of both micro- and macroorganisms. Humans have exploited these compounds

microbial origin have been characterized, with more than 60% produced by the gram positive

soil bacteria of the genus *Streptomyces* [(3)]. Of these, at least 70 are currently used for biomedical and agricultural applications. The largest class of bioactive compounds, the polyketides, include a broad range of antibiotics, immunosuppressants and anticancer agents which together account for sales of over $5 billion per year.

The paragraph at page 2, line 28 to page 3, line 10 was changed as follows:

Despite the seemingly large number of available bioactive compounds, it is clear that one of the greatest challenges facing modern biomedical science is the proliferation of antibiotic resistant pathogens. Because of their short generation time and ability to readily exchange genetic information, pathogenic microbes have rapidly evolved and disseminated resistance mechanisms against virtually all classes of antibiotic compounds. For example, there are virulent strains of the human pathogens *Staphylococcus* and *Streptococcus* that can now be treated with but a single antibiotic, vancomycin, and resistance to this compound will require only the transfer of a single gene, *vanA*, from resistant *Enterococcus* species for this to occur [(4)]. When this crucial need for novel antibacterial compounds is superimposed on the growing demand for enzyme inhibitors, immunosuppressants and anti-cancer agents, it becomes readily apparent why pharmaceutical companies have stepped up their screening of microbial diversity for bioactive compounds with novel properties.

The paragraph at page 3, lines 11-24 was changed as follows:

The approach currently used to screen microbes for new bioactive compounds has been largely unchanged since the inception of the field. New isolates of bacteria, particularly gram positive strains from soil environments, are collected and their metabolites tested for pharmacological activity. A more recent approach has been to use recombinant techniques to synthesize hybrid antibiotic pathways by combining gene subunits from previously characterized

[illegible] displayed activity [(5, 6)]. However, compounds with novel antibiotic activities have not yet

[illegible]

been reported, an observation that may be due to the fact that the pathway subunits are derived from those genes encoding previously characterized compounds. Dramatic success in using recombinant approaches to small molecule synthesis has been recently reported in the engineering of biosynthetic pathways to increase the production of desirable antibiotics [(7, 8)].

The paragraph at page 11, lines 17-20 was changed as follows:

In addition to protein-protein interactions, the study of the interaction of other molecules, and the ability to [effect] affect this interaction, is of interest in research and discovery processes and in the discovery of new drugs[. For] , for instance, steroids and their receptors, or polysaccharides and their receptors.

The paragraph at page 12, lines 15-21 was changed as follows:

In the present invention, for example, gene libraries generated from one or more uncultivated microorganisms are screened for an activity of interest. Potential gene pathways encoding bioactive molecules of interest are first captured in prokaryotic cells in the form of gene expression libraries and screened for activities of interest utilizing the methods of the present invention. Screening hosts can be modified to contain proteins or other molecules from metabolically rich cell lines which can aid in the expression of bioactive compounds such as small molecules.

The paragraph at page 16, lines 5-11 was changed as follows:

The microorganisms from which the libraries may be prepared include prokaryotic microorganisms, such as Eubacteria and Archaea, and lower eukaryotic microorganisms such as fungi, some algae and protozoa. Libraries may be produced from environmental samples in which case DNA may be recovered without culturing of an organism or the DNA may be

and the like.

The paragraph at page 24, lines 7-14 was changed as follows:

Since it appears that many bioactive compounds of bacterial origin are encoded in contiguous multigene pathways varying from 15 to 100 kbp [(11, 12)], cloning large genome fragments is preferred with the present invention, in order to express novel pathways from natural assemblages of microorganisms. Capturing and replicating DNA fragments of 40 to 100 kbp in surrogate hosts such as *E. coli*, *Bacillus* or *Streptomyces* is in effect propagating uncultivated microbes, albeit in the form of large DNA fragments each representing from 2 to 5% of a typical eubacterial genome.

The paragraph at page 24, lines 14-30 was changed as follows:

Two hurdles that must be overcome to successfully capture large genome fragments from naturally occurring microbes and to express multigene pathways from subsequent clones are 1) the low cloning efficiency of environmental DNA and 2) the inherent instability of large clones. To overcome these hurdles, high quality large molecular weight DNA is extracted directly from soil and other environments and vectors such as the f-factor based Bacterial Artificial Chromosome (BAC) vectors are used to efficiently clone and propagate large genome fragments. The environmental library approach [(Figure 1)] will process such samples with an aim to archive and replicate with a high degree of fidelity the collective genomes in the mixed microbial assemblage. The basis of this approach is the application of modified Bacterial Artificial Chromosome (BAC) vectors to stably propagate 100-200 kbp genome fragments. The BAC vector and its derivative, the fosmid (for f-factor based cosmid), use the f-origin of replication to maintain copy number at one or two per cell [(14)]. This feature has been shown to be a crucial factor in maintaining stability of large cloned fragments [(15)]. High fidelity replication is especially important in propagating libraries comprised of high GC organisms such

The paragraph at page 39, lines 21-28 was changed as follows:

FACS screening of clones using the methods of the present invention can be performed as described in U.S. Patent Application Number _____, [Filed] filed June 16, 1997. Other devices which utilize detectors capable of detecting any detectable molecule utilized in a method of the present invention may be employed. Such devices include, but are not limited to, a variety of high throughput cell sorting instruments, robotic instruments, and time-resolved fluorescence instruments, which can actually measure the fluorescence from a single molecule over an elapsed period of time.

The paragraph at page 45, lines 8-12 was changed as follows:

30 milliliters (ml) [ml] glass beads (Biospec Products, Bartlesville, OK) are mixed with 50ml APS/Toluene (10% APS) (Sigma Chemical Co.)
Reflux overnight
Decant and wash 3 times with Toluene
Wash 3 times with ethanol and dry in oven

The paragraph at page 45, lines 15-23 was changed as follows:

25 ml prepared glass beads from above
15 ml 0.1M NaHCO3 + 25 milligrams (mg) [mg] N-Acetyl-B-d-glucosamine-PITC (Sigma Chemical Co.) + 1 ml DMSO
Add 10 ml NaHCO3 + 1 ml DMSO
Pour over glass beads
Let shake in Falcon Tube overnight
Wash with 20 ml 0.1M NaHCO3
Wash with 50 ml ddH2O

The paragraph at page 46, lines 7-12 was changed as follows:

Place approximately 1-5mls of the derivitized beads into a Spectra/mesh nylon filter, such as those available from Spectrum (Houston, Texas) with a mesh opening of 70 μm, an open area of 43%, and a thickness of 70 μm. Seal the nylon filter to create a "bag" containing the beads using, for instance, Goop, Household Adhesive & Sealant.

The paragraph at page 47, lines 16-18 was changed as follows:

A. Digest 10 μl DNA with EcoRI restriction enzyme (Strategene Cloning Systems) according to manufacturers protocol electrophorese DNA digest through 0.5% agarose, 20V overnight; stain gel in 1 μg/ml EtBr

1. Determine DNA concentration (A_{260}-A_{280}).

The paragraph at page 47, lines 22-26 was changed as follows:

1. Incubate the following at 37°C for 3 hours:
   8 μl DNA (~10 μg)
   35 μl H_2O
   5 μ1 10x restriction enzyme buffer
   2 μl EcoRI restriction enzyme (200 units)

The paragraph at page 48, line 4 was changed as follows:

3. Examine 5 μl of each fraction on 0.8% agarose gel.

The paragraph at page 48, line 9 was changed as follows:

7. Dry, resuspend in 15 μl 5T 1E.

The paragraph at page 49, lines 3-5 was changed as follows:

\ Add 50 nanograms (ng) [ng] each of insert and vector DNA to 1U of T4 DNA ligate

The paragraph at page 49, line 19 to page 50, line 10 was changed as follows:

Cell collection and preparation of DNA. Agarose plugs containing concentrated picoplankton cells were prepared from samples collected on an oceanographic cruise from Newport, Oregon to Honolulu, Hawaii. Seawater (30 liters) was collected in Niskin bottles, screened through 10 μm Nitex, and concentrated by hollow fiber filtration (Amicon DC10) through 30,000 MW cutoff polysulfone filters. The concentrated bacterioplankton cells were collected on a 0.22 μm, 47 mm Durapore filter, and resuspended in 1 ml of 2X STE buffer (1M NaCl, 0.1M EDTA, 10 mM Tris, pH 8.0) to a final density of approximately 1 X 10^{10} cells per ml. The cell suspension was mixed with one volume of 1% molten Seaplaque LMP agarose (FMC) cooled to 40°C, and then immediately drawn into a 1 ml syringe. The syringe was sealed with parafilm and placed on ice for 10 min. The cell-containing agarose plug was extruded into 10 ml of Lysis Buffer (10 mM Tris pH 8.0, 50 mM NaCl, 0.1M EDTA, 1% Sarkosyl, 0.2% sodium deoxycholate, a mg/ml lysozyme) and incubated at 37°C for one hour. The agarose plug was then transferred to 40 mls of ESP Buffer (1% Sarcosyl, 1 mg/ml proteinase-K, in 0.5M EDTA), and incubated at 55°C for 16 hours. The solution was decanted and replaced with fresh ESP Buffer, and incubated at 55°C for an additional hour. The agarose plugs were then placed in 50 mM EDTA and stored at 4°C shipboard for the duration of the oceanographic cruise.

The paragraph at page 50, lines 11-26 was changed as follows:

One slice of an agarose plug <u>seventy-two microliters</u> (72 l) prepared from a sample collected off the Oregon coast was dialyzed overnight at 4°C against 1 ml of buffer A (100 mM NaCl, 10 mM Bis Tris Propane-HCl, 100 μg/ml acetylated BSA; pH 7.0 @ 25°C) in a 2 mL microcentrifuge tube. The solution was replaced with 250 μl of fresh buffer A containing 10 mM $MgCl_2$ and 1 mM DTT and incubated on a rocking platform for 1 hr at room temperature. The solution was then changed to 250 μl of the same buffer containing 4U of Sau3A1 (NEB),

68°C for 30 min to inactivate the protein, e.g., enzyme, and to melt the agarose. The agarose was

digested and the DNA dephosphorylased using Gelase and HK-phosphatase (Epicentre), respectively, according to the manufacturer's recommendations. Protein was removed by gentle phenol/chloroform extraction and the DNA was ethanol precipitated, pelleted, and then washed with 70% ethanol. This partially digested DNA was resuspended in sterile H_2O to a concentration of 2.5 ng/µl for ligation to the pFOS1 vector.

The paragraph at page 50, line 27 to page 51, line 9 was changed as follows:

PCR amplification results from several of the agarose plugs (data not shown) indicated the presence of significant amounts of archaeal DNA. Quantitative hybridization experiments using rRNA extracted from one sample, collected at 200 m of depth off the Oregon Coast, indicated that planktonic archaea in (this assemblage comprised approximately 4.7% of the total picoplankton biomass (this sample corresponds to "PACI"-200 m in Table 1 of DeLong *et al.*, High [high] abundance of Archaea in Antarctic marine picoplankton, *Nature*, *371*:695-698,1994)). Results from archaeal-biased rDNA PCR amplification performed on agarose plug lysates confirmed the presence of relatively large amounts of archaeal DNA in this sample. Agarose plugs prepared from this picoplankton sample were chosen for subsequent fosmid library preparation. Each 1 ml agarose plug from this site contained approximately 7.5×10^5 cells, therefore approximately 5.4×10^4 [5.4×10^5] cells were present in the 72 µl slice used in the preparation of the partially digested DNA.

The paragraph at page 51, lines 10-26 was changed as follows:

Vector arms are prepared from pFOS1 as described (Kim *et al.*, Stable propagation of cosmid sized human DNA inserts in an f-factor based vector, *Nucl. Acids Res.*, *20*:10832-10835, 1992). Briefly, the plasmid is completely digested with AstII, dephosphorylated with HK phosphatase, and then digested with BamHI to generate two arms, each of which contains a *cos*

reaction containing 25 ng each of vector and insert and 1U of T4 DNA ligase (Boehringer

Mannheim). The ligated DNA in four microliters of this reaction is *in vitro* packaged using the Gigapack XL packaging system (Stratagene), the fosmid particles transfected to *E. coli* strain DH10B (BRL), and the cells spread onto LB_{cm15} plates. The resultant fosmid clones are picked into 96-well microliter dishes containing LB_{cm15} supplemented with 7% glycerol. Recombinant fosmids, each containing ca. 40 kb of picoplankton DNA insert, have yielded a library of 3,552 fosmid clones, containing approximately 1.4×10^8 base pairs of cloned DNA. All of the clones examined contained inserts ranging from 38 to 42 kbp. This library is stored frozen at -80°C for later analysis.

The paragraph at page 52, lines 5-14 was changed as follows:

Sample composed of genomic DNA is purified on a cesium-chloride gradient. The cesium chloride (Rf = 1.3980) solution is filtered through a 0.2 μm filter and 15 ml is loaded into a 35 ml OptiSeal tube (Beckman). The DNA is added and thoroughly mixed. Ten micrograms of bis-benzimide (Sigma; Hoechst 33258) is added and mixed thoroughly. The tube is then filled with the filtered cesium-chloride solution and spun in a VTi50 rotor in a Beckman L8-70 Ultracentrifuge at 33,000 rpm for 72 hours. Following centrifugation, a syringe pump and fractionator (Brandel Model 186) are used to drive the gradient through an ISCO UA-5 UV absorbance detector set to 280 nm. Peaks representing the DNA from the organisms present in an environmental sample are obtained.

The paragraph at page 53, lines 4-16 was changed as follows:

Hybridization screening may be performed on fosmid clones from a library generated according to the protocol described in Example 3 above in any fosmid vector. For instance, the pMF3 vector is a fosmid based vector which can be used for efficient yet stable cloning in *E. coli* and which can be integrated and maintained stably in *Streptomyces coelicolor* or *Streptomyces*

identified by screening the library by colony hybridization using sequences designed from

previously published sequences of *oxy* and *tcm* genes. [}(27, }28)] Colony hybridization screening is described in detail in "Molecular Cloning," A Laboratory Manual, Sambrook, *et al.*, (1989) 1.90-1.104. Colonies that test positive by hybridization can be purified and their fosmid clones analyzed by restriction digestion and PCR to confirm that they contain the complete biosynthetic pathway. (See Figure 6).

The paragraph at page 54, lines 2-7 was changed as follows:

1 µl fosmid/insert DNA
5 µl each primer (50 ng/µl)
1 µl Boehringer Mannheim EXPAND Polymerase from their EXPAND kit
1 µl dNTP's
5 µl 10X Buffer #3 from Boehringer Mannheim EXPAND kit
30 µl ddH_2O

The paragraph at page 54, line 17 to page 55, line 11 was changed as follows:

Fosmid DNA from clones that are shown to contain the oxytetracyclin or tetracenomycin polyketide encoding DNA sequences are then used to transform *S. lividans* TK24 D*act* protoplasts from Example 6. Transformants are selected by overlaying regeneration plates with hygromycin (pMF5). Resistant transformants are screened for bioactivity by overlaying transformation plates with 2ml of nutrient soft agar containing cells of the test organisms *Escherichia coli* or *Bacillus subtilis*. *E. coli* is resistant to the thiostrepton concentration (50 mg/ml) to be used in the overlays of pMF3 clones but is sensitive to oxytetracyclin at a concentration of 5 mg/ml [(29)]. The *B. subtilis* test strain is rendered resistant to thiostrepton prior to screening by transforming with a thiostrepton marker carried on pHT315 [(30)]. Bioactivity is demonstrated by inhibition of growth of the particular test strain around the

methanol in a 1:1 ratio with the clone fermentation broth followed by overnight shaking at 4 C.

Cell debris and media solids in the aqueous phase are then separated by centrifugation. Recombinantly expressed compounds are recovered in the solvent phase and may be concentrated or diluted as necessary. Extracts of the clones are aliquoted onto 0.25-inch filter disks, the solvent allowed to evaporate, and then placed on the surface of an overlay containing the assay organisms. Following incubation at appropriate temperatures, the diameter of the clearing zones is measured and recorded. Diode array HPLC, using authentic oxytetracyclin and tetracenomycin as standards, can be used to confirm expression of these antibiotics from the recombinant clones.

The paragraph at page 55, lines 13-22 was changed as follows:

Sequence analysis of chromosomally integrated pathways identified by screening can be performed for confirmation of the bioactive molecule. One approach which can be taken to rescue fosmid DNA from *S. lividans* clones exhibiting bioactivity against the test organisms is based on the observation that plasmid vectors containing IS*117*, such as pMF3, are present as circular intermediates at a frequency of 1 per 10-30 chromosomes [(31)]. The presumptive positive clones can be grown in 25 ml broth cultures and plasmid DNA isolated by standard alkaline lysis procedures. Plasmid DNA preps are then used to transform *E. coli* and transformants are selected for Cm^r by plating onto LB containing chloramphenicol (15 mg/ml). Fosmid DNA from the *E. coli* Cm^r transformants is isolated and analyzed by restriction digestion analysis, PCR, and DNA sequencing.

IN THE CLAIMS

16. (Amended) A method for identifying a DNA sequence [molecule] which encodes a molecule or molecules which directly or indirectly modulate[s] the interaction between at least a

[illegible] detectable signal or growth of the cell, genomic DNA or clones of a DNA library generated from

nucleic acid derived from a mixed population of organisms and measuring the interaction of a first [hybrid protein] interacting molecule and a second [hybrid protein] interacting molecule in the presence of a [test] third molecule encoded by the library or the genomic DNA or produced as a result of expression of one or more products encoded by the library or the genomic DNA, wherein [the first hybrid protein comprises a first domain and the first protein and the second hybrid protein comprises a second domain and the second protein, wherein] interaction of the first [protein] and the second [protein causes] molecules in the absence of the third molecule produces a detectable [response] signal or growth of the cell; [and]

comparing the [detectable response] signal or growth of the cell in the presence and absence of the [test molecule] genomic DNA or library, wherein a difference between the response[s] or growth is indicative of [a test] the presence of a molecule that modulates [protein-protein] interaction between the first and second molecules; and

identifying a clone or DNA sequence which encodes a molecule or molecules which directly or indirectly modulates the interaction between the first and second molecules.

17. (Amended) The method of claim 16, wherein [the first domain is] at least one of the interacting molecules contains a DNA-binding moiety and [the second domain is] at least one of the interacting molecules contains a transcriptional activation or a transcriptional repressor moiety [and the detectable response is the expression of a detectable gene].

19. (Amended) The method of claim 17, wherein the DNA-binding moiety and the transcriptional activation moiety are derived from [a] different proteins.

20. (Amended) The method of claim [17] 16, wherein the detectable signal is produced from a gene [encodes] encoding a protein selected from the group consisting of β-galactosidase,

22. (Amended) The method of claim [17] 16, wherein the detectable signal is encoded by a gene [is] present in a host cell.

23. (Amended) The method of claim 22, wherein the host cell further comprises a first recombinant gene encoding the first [hybrid protein] molecule, a second recombinant gene encoding the second [hybrid protein] molecule, or a third recombinant gene encoding the [test] third molecule, wherein the first, second or third gene are expressed in the host cell.

25. (Amended) The method of claim 23, wherein the host cell contains the first, second and third genes.

27. (Amended) The method of claim 16, wherein the [test molecules] library is derived from an environmental [library] sample.

29. (Amended) The method of claim [27] 16 or 28, wherein the environmental library is derived from an environmental sample comprising uncultured microorganisms.

33. (Amended) The method of claim [27] 16 or [28] 23, wherein the library is created by obtaining an environmental sample, enriching the environmental sample for [prokaryotic] eukaryotic organisms and selecting against [eukaryotic] prokaryotic organisms, isolating nucleic acids from the enriched sample, fractionating the nucleic acids, and cloning the isolated nucleic acids into a vector.

36. (Amended) A method for [detecting the ability of] identifying a molecule [to] that affects the interaction between a first and second [protein] molecule, comprising:

population of organisms or in the presence of the library or genomic DNA [, the first hybrid protein comprising:

(a) a first domain and the first protein; and

the second hybrid protein comprising:

(b) a second domain and the second protein;]

wherein association of the first and second [hybrid proteins] molecules in the absence of the [test] third molecule results in the absence or presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the [test] third molecule with the detectable response in the absence of the [test] third molecule, wherein a difference in response is indicative of the presence of a molecule that affects the interaction between a first and second molecule.

38. (Amended) The method of claim 37, further comprising, prior to (i):
[(A)] providing a prokaryotic host cell containing the detectable gene; and
[(B)] providing a first gene [capable of being] expressed in the host cell, the first gene encoding the first [hybrid protein] molecule.

39. (Amended) The method of claim 38, further comprising, prior to (i):
[(C)] providing a second gene [capable of being] expressed in the host cell, the second gene encoding the second [hybrid protein] molecule.

40. (Amended) The method of claim 39, further comprising, prior to (i):
[(D)] providing a third gene [capable of being] expressed in the host cell, the third gene encoding the [test] third molecule.

41. (Amended) The method of claim 40, further comprising, prior to (i):
[(E)] introducing said first, second and third genes into the host cell; and
[(F)] allowing expression of the genes.

42. (Amended) The method of claim [41] 36, wherein the [first domain is] molecule contains a DNA binding domain and [the second domain is] a transcriptional activation domain.

43. (Amended) The method of claim [41] 36, wherein the [first and second domains form] interaction between the first and second molecules forms a transcriptional repressor.

44. (Amended) The method of claim [41] 36, wherein the third gene is derived from an environmental library.

45. (Amended) The method of claim [44] 36, further comprising, prior to (i):

[(G)] obtaining an environmental sample; and

[(H)] enriching the sample for prokaryotic organisms [and selecting against eukaryotic organisms].

46. (Amended) The method of claim 45, further comprising producing a normalized library, comprising [, prior to (i)]:

[(I)] isolating nucleic acids from said enriched environmental sample;

[(J)] fractionating the isolated nucleic acids;

[(K)] melting the recovered fractions and allowing subsequent reannealing; and

[(L)] amplifying any single-stranded nucleic acids present in the sample.

47. (Amended) The method of claim 46, further comprising generating an expression library, comprising [, prior to (i)]:

[(M)] inserting isolated nucleic acids [resulting from (I) or (L)] into an expression vector.

48. (New) A method for identifying a molecule that affects the interaction between a first and second molecule, comprising:

(i) contacting a first molecule with a second molecule wherein at least one of the first or second molecules are derived from a library made from a mixed population of organisms, wherein association of the first and second molecules in the presence of an unidentified molecule results in the presence of a detectable response by changing expression of a detectable gene or detectable gene product; and

(ii) comparing the detectable response in the presence of the inidentified molecule and the first and second molecules with the detectable response in the absence of the unidentified molecule, wherein a difference in response is indicative of a first and second molecule that